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The Effect of Chemical Warfare Agents on the Immune System of Survivors in Halabja

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Abstract

To evaluate the incidence of immunocompetence, including cell-mediated and antibody fitness, among survivors of the chemical bombardment of Halabja in the Kurdistan region of Iraq, forty exposed and forty unexposed subjects regarded as controls were studied to determine their immune system status 12 years after bombardment. Skin reactivity to tuberculin, D.T.P. vaccine, T.T toxoid and measles vaccine was negative in 62.5% of the exposed cases in compare to unexposed persons who showed no negative reactions 0%. The total leukocyte count was normal among 70% of exposed cases, whereas the total lymphocyte count was within sub-normal ranges in 80% of exposed cases. All the subjects displaying negative skin reactions had sub-normal lymphocyte counts, which reflect impaired cell-mediated immunity. The immunoglobulin assay for exposed cases revealed sub-normal values for IgG (12.5%) and IgA (52.5%), while the IgM level was above the normal range in 22.5% of cases when compared to that of controls that showed no abnormal values. This result revealed that there was a deficiency in antibody-mediated immunity. There were significant differences between the exposed and the control samples with respect to total leukocytes ($p = 11\times 10^{-5}$), neutrophil count ($p = 0.88 \times 10^{-3}$), lymphocyte count ($p = 0.0$), IgG ($p = 0.74 \times 10^{-10}$) and IgA ($p = 0.1 \times 10^{-10}$). The immunological reactions were more closely related to the effects of mustard gas, which appeared to be long lasting.

Keywords: Chemical warfare; Immune system; Immunocompromised patients; Immunocompetence; Cell-mediated immunity; Antibody-mediated immunity

Introduction

Throughout history, innocent people have been used as targets for several kinds of weapon. These weapons include chemical warfare agents, which have a long history of use. These agents are groups of poisonous chemical compounds. They are lethal and toxic for human beings and other creatures that are exposed to them. One of these chemicals is Sulphur Mustard (SM), an oily liquid that vaporises slowly at climate temperatures and can be used in aerosol form by spraying or by explosive blasts [1]. Warfare agents were first used as chemical weapons by the German army in 1917 near Ypres, Belgium. During the First World War, SM caused the most [2] chemical casualties and has since been used in at least twelve conflicts, most recently in the Iran-Iraq war [3]. The Kurdistan region of Iraq was another experimental field for the effect of chemical weapons on humans in the 1980s during the Iran-Iraq war and in 1988 in particular.

The affected areas were several cities and villages 4].

The town of Halabja, which is located in the Kurdistan region of Iraq, is close to the Iraq-Iran border and about 260 km north east of Baghdad. This was the principal town to suffer from chemical bombardment by the Iraqi army. More than 5,000 people were

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killed and 10,000 were injured at the time of the actual bombardment, 16 March 1988.

The immune system is reported to be affected by exposed to SM. The earliest evidence came from clinical observations of humans directly exposed to this agent during the First World War. They displayed significant quantitative and qualitative changes in the circulating elements of the immune system. Stewart (1918) studied a group of cases of mustard poisoning and observed a striking depression in the bone marrow production of white blood cells [5]. Krumbhaar (1919) reported that one of the first changes in the circulating blood of people exposed to SM was the exhaustion of leukocyte-forming centres [6]. Similar observations were made by [7]. Alexander (1947) suggested that the effects of SM on the leukocytes in the circulating blood were most severe. Lymphocytes were the first to disappear and granulocytes were also severely affected but lagged behind the lymphocytes in their rate of decrease [8]. It is known that SM and nitrogen mustard have a special affinity for haematopoitic tissues; this could be responsible for suppressing antibody production in experimental animals exposed to these agents [9]. Infection was a dominant feature, just as it was among SM causalities during the Iraq-Iran conflict in 1980-1988. They experienced leukopenia accompanied by total bone marrow aplasia, which included extensive losses of myeloid stem cells [10, 11]. In one of the few studies of long-term effects, Zandieh *et al*. (1990) measured the cell-mediated immunity of three groups of Iranians exposed to SM; three months to two years, one to two years and more than two years after exposed [12]. Several recent case reports from the Bahar Medical Laboratory in Teheran, Iran, described similar long-term effects. The investigators found changes in B and T lymphocytes as a result of SM exposed [10]. In another study, Smith *et al.*. (1995) observed that high doses of SM can destroy firstly the peripheral white blood cells and then the bone marrow [13].

This study aimed to investigate the incidence of immunocompetence among survivors of chemical exposed in Halabja, including cell-mediated and antibody fitness.

Materials and methods

The study comprised eighty volunteers from the inhabitants of Halabja in between May and November 2000. Forty of them were survivors of the chemical attack on this city. They were suffering from a variety of health problems of varying severity. Their ages ranged from 16-69 years. The other 40 were people who had not been exposed to chemicals and had not been present inside the city at the time of bombardment and had left the city for more than two years after the bombing. Their age ranged between 25-47 years and they were regarded as negative controls. Participants in this study were selected randomly from different parts of the city and included both genders.

Blood samples were collected from exposed and unexposed cases. From each case, 5 ml. of sterile blood were taken (venous blood), using sterile syringes (5 cc). When the blood clotted, it was centrifuged at 5000 rpm. for 10 minutes. Serum was collected in a small tube with a plastic cap and stored at -20^{o}C for three months. The total leukocyte count and differential white blood cell count were also calculated according to [14]. Four different antigens were used to assess cell-mediated immunity;[15,16], they included tetanus toxoid (T.T) (Swiss Serum and Vaccine Institute), D.T.P vaccine (Pasteur Merieux), measles vaccine (Pasteur Merieux) and tuberculin (Institute Merieux). Physiological saline was used as the control. Both T.T and D.T.P were diluted 1:10 with physiological saline. In addition, live measles vaccine was killed by placing the vaccine in a water bath at 55^{o}C for 30 minutes. All the agents were injected intradermally into the forearm using a syringe. The tested area was cleaned using

antiseptic alcohol and the needle was inserted into the skin and channelled for several millimetres through the dermis, by moving the tip of the needle. A volume of 0.1 ml. antigen was injected into the skin. This procedure was repeated for each antigen. The result of this test (induration) was recorded at 48 and 72 hours and tabulated [15].

To test the immunoglobulin-mediated immunity, serum IgA, IgG and IgM levels were measured using the immunoturbidometric method [17, 18]. A working reagent was made by diluting anti-IgA, IgG and IgM with polyethylene glycol buffer (Spin react) 1:41 (0.5 ml of antiserum with 20 ml. of the buffer). A serum sample from each case was diluted with physiological saline 1:21 (0.5 ml. of the sample with 10 ml. of physiological saline). To prepare standard dilutions (working calibrator), physiological saline was used as a diluent to produce a serious dilution of the standard 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 and 1:128 as follows. Seven small test tubes (each containing 1 ml. of physiological saline) were placed in a test-tube rack and labelled (1 to 7). To the first tube, 1 ml of the standard was added (1:2) and, from the first tube (No. 1), 1 ml. was transferred to the second tube (No. 2) (1:4) and so on for the other tubes, until seven dilutions had been obtained. The Quick-Lab 2 Chemistry Analyzer (Ames) is computerised and programmed for several analytical parameters, including immunoglobulins. The calculations are made automatically. The same procedures as those previously described were used, beginning with the lowest concentration (1:128), followed by all the other concentrations. After the seventh concentration, diluted serum samples were used instead of calibrator dilutions. Prior to these procedures, the wavelength of the instrument was adjusted to 340 nm and the calibrator concentration was entered. The instrument automatically measured the immunoglobulin concentration of serum samples in mg/dl.

Results

The induration diameters of the skin tests after 72 hours are summarised in Table 1. Twenty-five exposed cases (62.5%) displayed no skin reactivity to any antigens. The other 15 (37.5%) displayed reactivity to one or more antigens, nine (60%) to DTP vaccine alone, two (13.33%) to tuberculin alone and the other four (26.67%) displayed reactivity to both tuberculin and DTP vaccine, while all the unexposed cases (100%) displayed reactivity to one or more antigens. Eight (53.33%) displayed reactivity to tuberculin and DTP vaccine, two (13.33%) to tuberculin, DTP and TT vaccines and two (13.33%) to DTP and TT vaccines, one (6.67%) to DTP alone and one (6.67%) to tuberculin, DTP and measles vaccines.

The total and differential leukocyte counts are summarised in Table 2. Eleven exposed cases (27.5%) were within the sub-normal total leukocyte count, ranging between 3,000 and 3,900 cells/mm^3. Only one (2.5%) had a high total leukocyte count (12,000 cells /mm^3). The other twenty-eight (70%) were within the normal leukocyte count, while all the unexposed cases had a normal leukocyte count, apart from three (7.5%) who had a low level (3,100 cells/mm^3). Nine exposed cases (22.5%) were within the sub-normal neutrophil levels, ranging between 1.5-1.92 $x10^9$/L. Only one (2.5%) had a high neutrophil level (9.8 $x10^9$/L). The other ten (75%) had normal levels, while all the unexposed cases were within normal neutrophil levels, apart from three (7.5%) who had a low level (1.79 $x10^9$, 1.75$x10^9$ and 1.78$x10^9$/L). Thirty-two exposed cases (80%) were within the sub-normal lymphocyte count, ranging between 0.59-1.45$x10^9$/L. Eight (20%) were within the normal lymphocyte count. Among the unexposed cases, four (10%) had a low lymphocyte count (0.96, 1.35, 0.98 and 9.7 $x10^8$/L), while the others (90%) were within the normal range. No abnormal

monocyte counts were seen among either exposed or unexposed cases; they were all within normal levels. All the exposed cases were within the normal eosinophil range, apart from one (2.5%) who had a low level (0.02 $x10^9$/L), while all the unexposed cases (100%) were within the normal eosinophil range. Basophils were only detected in 18 exposed cases (45%) which were within the normal range, while they were detected in ten unexposed cases (25%) that were within the normal range. Using Student's t test, significant differences were observed between the total leukocyte ($p = 0.11 \times 10^{-5}$), neutrophil ($p = 0.88 \times 10^{-3}$) and lymphocyte counts ($p = 0.0$) of exposed and unexposed cases. No significant differences were observed between either unexposed or exposed cases or among exposed cases when it came to monocyte, eosinophil and basophil counts.

The serum immunoglobulin levels of exposed and unexposed cases are summarised in Table 3. Five exposed cases (12.5%) were within sub-normal IgG levels, ranging from 546-783 mg/dl, while the other 35 (87.5%) were within normal IgG levels. All 40 unexposed cases (100%) had normal IgG levels. Twenty-one exposed cases (52.5%) were within sub-normal IgA levels, ranging from 80-108 mg/dl, while no abnormal IgA values were observed among unexposed cases. Nine exposed cases (22.5%) had higher IgM levels than normal, ranging from 230-580 mg/dl, while no abnormal IgM levels were seen among unexposed cases.

Using Student's t-test, significant differences were seen between exposed and unexposed cases in terms of the mean IgG ($p = 0.74 \times 10^{-10}$) and IgA ($p = 0.1 \times 10^{-10}$) levels, while the mean values for exposed serum IgM were higher than those of unexposed cases, but the difference was not significant (Fig. 1)

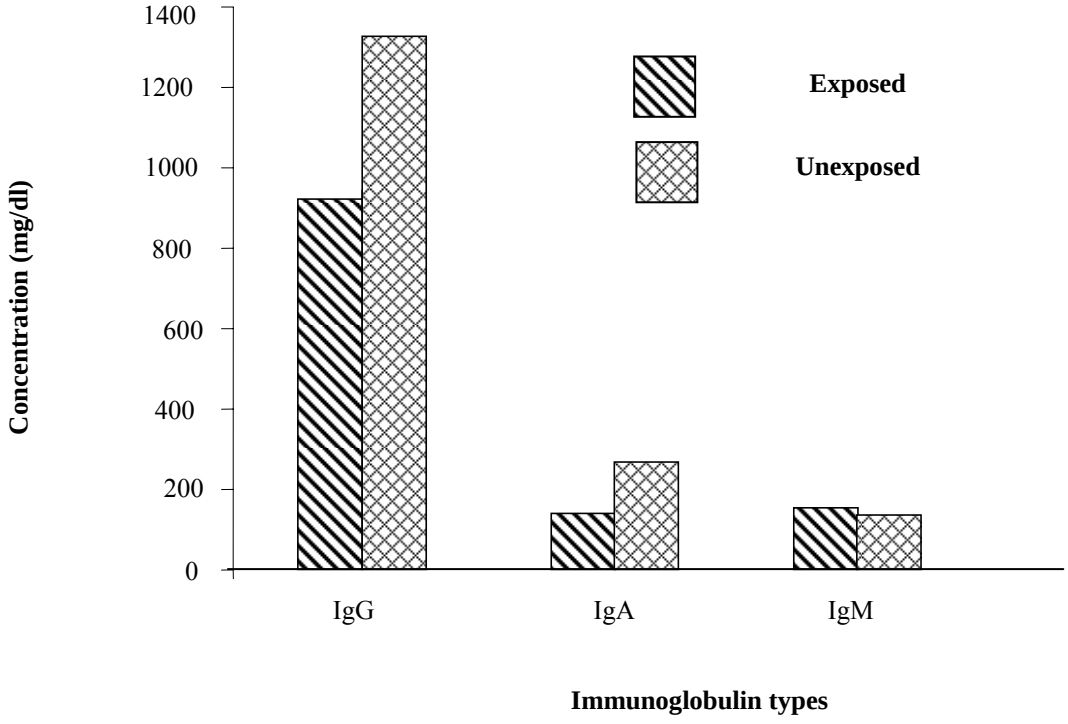

Figure-1- Serum Immunoglobulin levels of both Exposed and unexposed (mg/dl)

Table -1- Delayed type skin reaction tests for both exposed and unexposed after (72) hours of intradermally injection of some antigens*

Exposed						Non- Exposed					
Case No.	N.S	Tub.	D.T.P	T.T	Measles	Case No.	N.S	Tub.	D.T.P	T.T	Measles
47	-	-	+	-	-	1	-	++	+++	-	-
23	-	-	-	-	-	3	-	++	+++	+	-
31	-	-	+	-	-	4	-	+	+++	-	-
33	-	-	-	-	-	5	-	+++	+++	-	+++
38	-	-	-	-	-	6	-	-	+++	-	-
48		-	-	-	-	7	-	+++	+++	-	-
15	-	-	-	-	-	8	-	+	-	-	++
10	-	-	+	-	-	9	-	+	+++	-	-
19	-	-	-	-	-	42	-	-	+++	++	-
20	-	-	-	-	-	44	-	-	+	-	-
36	-	-	+	-	-	50	-	-	+	-	-
51	-	+	-	-	-	52	-	++	++	-	-
12	-	-	-	-	-	53	-	++	++	+	-
17	-	-	-	-	-	54	-	-	++	+	-
21	-	-	-	-	-	55	-	+++	++	-	-
40	-	-	-	-	-	56	-	+	+	-	-
43	-	-	-	-	-	57	-	-	+++	++	-
24	-	-	+	-	-	58	-	-	+++	-	-
29	-	-	-	-	-	59	-	+	-	-	++
22	-	-	-	-	-	60	-	+	+	-	-
25	-	-	-	-	-	61	-	+	+++	-	-
32	-	++	+	-	-	62	-	++	++	-	-
35	-	-	-	-	-	63	-	-	++	+	-
45	-	-	-	-	-	64	-	+++	++	-	-
46	-	-	-	-	-	65	-	+	+++	-	-
16	-	-	-	-	-	66	-	++	+++	+	-
18	-	-	-	-	-	67	-	++	+++	-	-
14	-	-	+	-	-	68	-	+++	+++	-	+++
37	-	-	-	-	-	69	-	+++	+++	-	-
39	-	-	+	-	-	70	-	++	++	+	-
13	-	-	-	-	-	71	-	+	+	-	-
30	-	+	-	-	-	72	-	-	+++	++	-
41	-	++	++	-	-	73	-	-	+++	-	-
26	-	-	-	-	-	74	-	+	-	-	++
28	-	-	+	-	-	75	-	+	+	-	-
2	-	++	++	-	-	76	-	++	++	-	-
11	-	-	-	-	-	77	-	-	++	+	-
27	-	-	+++	-	-	78	-	+++	++	-	-
34	-	-	-	-	-	79	-	+++	+++	-	+++
49	-	+	++	-	-	80	-	+++	+++	++	-

* N.S = Normal saline Tub. = Tuberculin D.T.P. vaccine T.T = Tetanus toxoid, and Measles vaccine.

\+ Low reactivity ++ Medium reactivity +++ High reactivity

Table -2- Leukocyte counts of both exposed and unexposed.

Exposed							Non- Exposed						
Case No.	Leukocytes count						Case No.	Leukocytes count					
	Total (Cells/mm^3) X100	Differential count x10^9						Total (Cells/mm^3) X100	Differential count x10^9				
		Neut.	Lym ph.	Mon o.	Eos.	Bas			Neut.	Lym ph	Mon o.	Eos.	Bas
47	36	2.34	0.82	0.28	0.14	Nd	**1**	68	4.20	2.00	0.34	0.20	Nd
23	39	1.79	1.44	0.54	0.12	Nd	**3**	78	4.68	2.26	0.54	0.30	Nd
31	50	2.70	1.60	0.45	0.25	Nd	**4**	62	3.90	1.73	0.37	0.24	0.06
33	30↓	1.50↓	1.20↓	0.21	0.06	Nd	**5**	82	4.18	2.54	0.57	0.32	Nd
38	120↑	9.80↑	1.20↓	0.72	0.24	Nd	**6**	70	4.27	2.00	0.42	0.20	0.07
48	35↓	2.45	0.59↓	0.24	0.17	0.03	**7**	73	4.52	2.19	0.36	0.21	Nd
15	46	2.76	1.15↓	0.27	0.36	0.04	**8**	31	1.79	0.96	0.28	0.12	Nd
10	40	2.20	1.16↓	0.44	0.20	Nd	**9**	50	3.00	1.35	0.45	0.15	0.05
19	36↓	1.8↓	0.90↓	0.46	0.39	0.03	**42**	70	4.40	2.00	0.35	0.21	Nd
20	35↓	1.92↓	0.87↓	0.45	0.21	0.03	**44**	42	2.18	1.59	0.25	0.12	0.04
36	41	2.23	1.50	0.32	0.16	0.04	**50**	76	4.00	2.50	0.68	0.38	Nd
51	53	3.28	1.06↓	0.53	0.37	0.05	**52**	55	3.24	1.70	0.38	0.16	Nd
12	42	2.22	1.05↓	0.67	0.21	0.04	**53**	68	4.42	1.76	0.40	0.20	Nd
17	48	2.64	1.24↓	0.57	0.28	0.04	**54**	71	4.04	2.05	0.70	0.21	Nd
21	44	2.15	1.18↓	0.62	0.40	0.04	**55**	59	3.90	1.55	0.23	0.11	Nd
40	46	3.31	0.73↓	0.40	0.13	Nd	**56**	42	2.17	1.60	0.26	0.13	0.04
43	3↓	1.62↓	0.81↓	0.42	0.12	0.03	**57**	70	4.35	2.05	0.34	0.22	Nd
24	43	2.45	1.38↓	0.30	0.17	Nd	**58**		4.28	2.04	0.40	0.23	0.06
29	40	2.24	0.92↓	0.60	0.20	0.04	**59**	31	1.78	0.98	0.22	0.12	Nd
22	41	2.09	1.18↓	0.53	0.24	0.04	**60**	76	4.01	2.52	0.65	0.35	Nd
25	48	3.26	0.96↓	0.38	0.19	Nd	**61**	50	3.02	1.36	0.40	0.16	0.05
32	68	3.52	1.70	0.63	0.37	0.06	**62**	55	3.25	1.75	0.35	0.17	Nd
35	60	4.20	1.20↓	0.42	0.18	Nd	**63**	71	4.05	2.08	0.65	0.22	Nd
45	47	3.24	0.98↓	0.28	0.18	Nd	**64**	59	3.91	1.54	0.25	0.12	Nd
46	50	3.65	0.80↓	0.30	0.25	Nd	**65**	62	3.92	1.72	0.36	0.23	0.05
16	36↓	1.80↓	1.08↓	0.50	0.18	0.03	**66**	78	4.67	2.25	0.55	0.29	Nd
18	40	2.40	1.10↓	0.28	0.20	0.04	**67**	68	4.25	2.04	0.33	0.21	Nd
14	40	2.20	1.50	0.20	0.12	Nd	**68**	82	4.17	2.55	0.58	0.33	Nd
37	48	2.50	0.80↓	0.53	0.14	0.04	**69**	73	4.52	2.20	0.34	0.22	Nd
39	50	3.05	1.60	0.23	0.12	Nd	**70**	68	4.43	1.75	0.42	0.21	Nd
13	40	2.50	0.92↓	0.36	0.21	Nd	**71**	42	2.19	1.60	0.29	0.13	0.05
30	50	2.90	1.50	0.35	0.20	0.05	**72**	70	4.43	2.03	0.33	0.25	0.07
41	50	3.00	1.45↓	0.40	0.20	Nd	**73**	70	2.28	2.00	0.46	0.18	Nd
26	60	4.80	0.60↓	0.24	0.36	Nd	**74**	31	1.75	0.97	0.22	0.13	Nd
28	50	3.00	1.35↓	0.50	0.15	Nd	**75**	76	4.05	2.6	0.67	0.36	Nd
2	35↓	1.90↓	1.60	0.45	0.17	0.03	**76**	55	3.25	1.8	0.35	0.12	Nd
11	32↓	1.79↓	0.89↓	0.35	0.16	Nd	**77**	71	4.05	2.06	0.71	0.23	Nd
27	68	4.76	1.56	0.47	0.20	Nd	**78**	59	3.85	1.53	0.22	0.12	Nd
34	46	2.30	1.28↓	0.59	0.36	0.04	**79**	82	4.20	2.50	0.55	0.33	Nd
49	36↓	1.90↓	1.40↓	0.21	0.02	Nd	**80**	73	4.50	2.21	0.35	022	Nd

Nd = not detected ↑ above the normal range ↓ below the normal range neut = neutrophils lymph = lymphocytes Mono = monocytes eios = eiosinophils bas = basophils

WBC sig. diff — assuming equal variances: t = -5.27807 P-value = 0.00000114225

Lym sig. diff — assuming equal variances: t = -8.97541 P-value = 0.0

Neut sig. diff — assuming equal variances: t = -3.45634 P-value = 0.000889215

Mon not sign. — assuming equal variances: t = 0.0935705 P-value = 0.92569

Eios not sign. assuming equal variances: t = -1.00033 P-value = 0.320242

Table -3- Serum Immunoglobulin levels of both exposed and unexposed*

Exposed				Non- Exposed			
Case No.	Immunoglobulin levels (mg/dl)			Case No.	Immunoglobulin levels (mg/dl)		
	IgG	IgA	IgM		IgG	IgA	IgM
47	959	103↓	159	**1**	1010	380	96
23	870	80↓	411↑	**3**	1250	267	190
31	783↓	93↓	85	**4**	1630	168	140
33	925	102↓	107	**5**	990	254	94
38	546↓	90↓	109	**6**	1198	270	170
48	1038	165	89	**7**	1027	145	112
15	821	100↓	230	**8**	1740	410	139
10	830	100↓	180	**9**	995	195	86
19	920	88↓	120	**42**	1435	315	130
20	1083	107↓	136	**44**	1800	375	210
36	811	91↓	230↑	**50**	1680	267	183
51	1200	320	112	**52**	1120	213	103
12	803	89↓	137	**53**	1720	342	208
17	900	137	81	**54**	931	243	93
21	720↓	101↓	232↑	**55**	1048	169	87
40	813	170	300↑	**56**	1750	375	211
43	1085	142	92	**57**	1430	320	135
24	900	95↓	120	**58**	1190	272	165
29	781↓	85↓	93	**59**	1750	415	136
22	890	301	73	**60**	1675	268	184
25	900	105↓	55	**61**	992	196	82
32	1046	203	94	**62**	1130	215	105
35	880	114	54	**63**	920	245	98
45	826	114	47	**64**	1550	165	88
46	870	93↓	160	**65**	1620	164	138
16	1010	130	89	**66**	1255	270	188
18	830	83↓	238↑	**67**	1520	375	95
14	842	95↓	126	**68**	995	255	98
37	913	98↓	87	**69**	1030	149	113
39	930	108↓	580↑	**70**	1725	348	207
13	765↓	93↓	102	**71**	1759	374	211
30	927	190	133	**72**	1440	312	132
41	1134	190	110	**73**	1189	271	172
26	1150	220	98	**74**	1750	412	135
28	985	142	255↑	**75**	1655	265	181
2	1047	207	295↑	**76**	1125	215	106
11	927	170	143	**77**	935	241	95
27	1065	260	170	**78**	1050	168	88
34	998	185	76	**79**	994	255	95
49	1108	213	121	**80**	1030	144	114

↑ above the normal range ↓ below the normal range * Normal ranges are according to Roit, M. (1997).

IgG sig diff.

assuming equal variances: t = -7.5362 P-value = 7.40472E-11

IgA sig diff.

assuming equal variances: t = -7.96885 P-value = 1.08074E-11

IgM no sig diff.

assuming equal variances: t = 1.0055 P-value = 0.317763

Discussion

According to the WHO Scientific Group (1995), the positive results of delayed-type hypersensitivity skin tests (induration) appearing after 72 hours reflect intact cell-mediated immunity. This test is an *in vitro* T-lymphocyte functional assay [16]. On the other hand, negative skin reactivity indicates impaired cell-mediated immunity [15]. This leads to the conclusion that the differences between exposed and unexposed cases indicate impaired cell-mediated immunity among survivors. The observations reported by Zandieh *et al.*. (1990), when they investigated the cell-mediated immunity of three groups of Iranian chemical victims, are similar to our results [12]. They found a significant reduction in the peripheral blood T-lymphocytes in most of their patients and noted impaired cell-mediated immunity. It should be noted that the Iranian and Halabja victims were exposed to the same source of warfare agents by the Iraqi army. Similar observations were made by the Bahar Medical Laboratory in Teheran when it studied the long-term effects of SM on a number of Iranian victims [10]. Blank *et al.*. (1991) found that mustard compounds induced splenic and thymic weight reduction in mice, leading to impaired cell-mediated immunity [19]. It is therefore likely that negative skin reactivity among exposed cases could reflect the noxious effect of SM on lymphocyte production and function. This may explain the reduction in the total lymphocyte count in the peripheral blood of the exposed cases, which was apparently related to negative skin reactivity, as all the exposed cases which had negative skin tests had reduced or sub-normal lymphocyte counts.

The wide range of changes seen in the total leukocyte count of survivors might be due to the reduction in neutrophils or lymphocytes that comprise a large ratio of total leukocytes. As a result, exposed cases with a low total leukocyte count were suffering from neutropenia, sub-normal lymphocytes, or both. Several factors may cause leukopenia; they include a reduced flow of leukocytes (neutrophils) from the bone marrow into the peripheral blood, (due to lack of production or ineffective production), the increased removal of leukocytes from the blood, a change in distribution between the circulating granulocyte pool and the marginal granulocyte pool and sometimes a combination of these factors [14].

Exposed to chemical warfare agents including mustard compounds may be the main factor that leads to low leukocyte levels, because of the effect these compounds have on bone marrow. Anslow and Houk (1946) reported leukopenia and loss of bone marrow reactivity in severe cases of mustard intoxication in animals [7]. They also suggested that similar effects had occurred in soldiers gassed during the First World War. Alexander (1947) reported the effect of SM on the leukocytes in the circulating blood of humans and found severe toxic effects causing leukopenia [8]. He also noted that lymphocytes were the first to disappear, followed by granulocytes, which were severely affected. The intravenous injection of mustard compounds in albino rats led to reduced immunoresponsiveness expressed as leukopenia, lymphocytopenia and neutropenia, as well as the hypoplasia and hypermia of bone marrow [20].

Dean and Murray (1991) concluded that SM is a leukocytic toxin acting on the bone marrow with myelotoxicity causing leukopenia, pancytopenia, anemia and plastic or hypoplastic bone marrow in experimental animals [21]. The reduction in the total leukocyte, neutrophil and lymphocyte counts among chemical survivors in Halabja may therefore be due to the severe effects of long-term exposed to mustard compounds, although 12 years have passed since the chemical bombardment. This also confirms the long-lasting effects of these compounds.

Significant differences were noted between the serum immunoglobulin levels of exposed and unexposed cases, especially those of IgG and IgA, and this may indicate that there were

deficiencies in antibody–mediated immunity, because the two above-mentioned immunoglobulins play important roles in this type of immunity. The low levels of immunoglobulin were the result of low B-cell levels and deficiencies in the function and differentiation of antibody-producing plasma cells [16].

Chemical warfare agents are toxic chemical compounds which have large-scale effects on different organs and tissues. SM is one of the toxic compounds that were used against the innocent inhabitants of Halabja [4]. It has a direct toxic effect on haematopoitic tissues in the bone marrow, [13, 22] which is regarded as one of the primary immunopoitic organs. The toxic effects of SM have been confirmed in experimental animals [20, 23, 24]. Anslow and Houck (1946) confirmed the effect of SM on humans during the First World War [7]. The action of SM on immunopoitic centres may lead to defects in immune cell production. It has been shown that B-lymphocytes were relatively more severely affected than T-lymphocytes due to high doses of SM leading to a reduction in B-cell numbers following exposed to that agent [25]. Further studies reported the suppression of antibody production in experimental animals due to mustard compounds [9, 24]. So the reductions in the serum immunoglobulin levels of survivors may be due to the action of chemical warfare agents, including SM, which adversely affect B-cells.

According to medical reports on exposed cases that were examined by doctors specialising in clinical illnesses (not included in this study), some of the survivors suffer from allergy problems, particularly in the respiratory tract, which may be related to IgA levels [26], as well as other severe respiratory, dermatological and ophthalmological complaints recorded in their medical reports. Infante and Kamani (1997) mentioned that reduced IgA levels may sometimes be accompanied by IgG sub-class deficiencies, in spite of normal total IgG levels [7]. They also reported that the majority of IgA-deficient individuals have recurrent sinusitis and minor upper-respiratory tract infections and they have shown that patients with IgG sub-class deficiency suffer from significant respiratory infections [27]. Moreover, low immunoglobulin levels may be due to chronic secondary bacterial infections, following injuries caused by exposed to chemical warfare agents. Some cases among survivors with elevated IgM levels may have been due to the direct or indirect action of chemical warfare agents on B-lymphocytes,[25] or they may have been due to the defects that prevent B-cells from switching IgM to IgG and IgE [28]. It is known that common clinical manifestations, including individuals with upper- and lower-respiratory tract infections, are related to elevated IgM levels [29]. This may explain the elevated IgM levels among some of the people exposed to chemicals. The reduced or elevated (abnormal) immunoglobulin levels among survivors may be due to the direct or indirect action of chemical agents used in the attack, precisely because the changes were only seen among survivors and not among controls (unexposed cases). Further more the results ensured other conclusions pointed to the lethal and chronic effects of the chemical warfare agents and the long lasting effects on human health and on the life in general [30-32].

The main conclusion from this study is that long-term effects were produced by chemical warfare agents on victims who have survived in Halabja and, in particular, on their immune system at both antibody and cell-mediated levels. This confirms the immunosuppressive property of mustard compounds, which may lead to the appearance of secondary opportunistic or pyogenic bacterial infections due to injuries which occurred during the attack and impaired immunity that has been frequently observed among chemical survivors in Halabja.

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کاریگەریەکانی چەکە کیمیاویەکان لە سەر کۆئەندامی بەرگری بەرکەوتوانی ڕزگاربوو لە هەڵبجە

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پوختە

لە پێناو خەملاندنی توانستی کۆئەندامی بەرگری لە سەر هەردوو ئاستی دژەتەنە بەرگری و خانە بەرگری لە نێوان رزگاربوان لە کیمیابارانی هەڵبجە کە دەکەوێتە هەرێمی کوردستانی عێراق, چل کەسی بەرکوتوو وە چل کەسی بەرنەکەوتوو کە وەك کۆنترۆل دانران تاقیکرانەوە بە مەبەستی بەدیارخستنی باری کۆئەندامی بەرگرییان دوای 12 ساڵ لە بۆمباران. کارلێکی پێست بەرانبەر گوتاوەکانی tuberculin , تۆکسۆیدی تیتانۆس D.T.P, Tetanus toxoid و سورێژە نە نجامی نێگەتیفی نیشاندا لە نێوان (62.5٪) ی کەسە بەرکەوتوەکان بەبەراورد لە گەڵ بەرنەکەوتوان کە کەسیان نە نجامی سالبیان نەبوو (0.00٪). ژمارەی گشتی خرۆکەسپیەکان لە مەودای ئاسایی بوو لە نێوان 70٪ ی بەرکەوتواندا, لەکاتێکدا ژمارەی لیمفەخانەکان لە ئاستی ئاسایی خۆی نزمتر بوو لە نێوان 80٪ ی بەرکەوتوان . سەرجەمی ئەوانەی کە تاقیکردنەوەی پێستیان نێگەتیف بوو ژمارەی لیمفە خانەکانیان لە ژێر ئاستی ئاساییەوە بوو, ئەمەش نیشانی دەدات کە ئەو ڕێژەیەی ی بەرکەوتوان گرفتی کەمبوونەوەی ئەو جۆرە بەرگریەیان هەیە کە خانەکان لێی بەرپرسن.

ئەژمارکردنی دژەتەنکان بۆ بەرکەوتوان بەدەریا نخست کە 12.5٪ (IgG) و 52.5٪ ی (IgA) لە ئاستی ئاسایی خۆیان نزمترن بەڵام (IgM) لە 22.5٪ ی بەرکەوتوان لە ئاستی ئاسایی بەرزتر بوو لەکاتێکدا سەرجەم ئە نجامی بەرنەکەوتوان ئاسایی بوون. ئەم ئە نجامانە دەریدەخەن کە کەموکوڕیی بەدیدەکرێت لە و خۆرە بەرگریەی دژەتەنەکان لێی بەرپرسن. جیاوازی بەرچاو هەبوو لە نێوان بەرکەوتوان و بەرنەکەوتوتاندا بە بەرچاو گرتنی ژمارەی گشتی خرۆکە سپیەکان ($p = 1.1 \times 10^{-4}$) , هاوتاخانەکان ($p = 8.8 \times 10^{-2}$) , لیمفەخانەکان ($p = 0.0$) , IgG ($p = (7 \times$) و IgA ($p = 1 \times 10^{-10}$) . کارلێکەکان لەسەر کۆئەندامی بەرگری بەکارهێنانی گازی خەردەل دەسەلێنن کە دەردەکەوێت کاریگەری درێژخایەنی هەیە.

تأثيرات الاسلحة الكيمياوية على جهاز المناعة للمتعرضين الناجين فى حلبجة

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الخلاصة

لغرض قياس كفاءة الجهاز المناعى عند مستوى المناعة الضدية و الخلوية للناجين من القصف الكيمياوى لمدينة حلبجة الواقعة الى الشرق من اقليم كردستان العراق , اجريت تجارب على (40) متعرض و (40) غير متعرض –عينة السيطرة- بعد مرور 12 عاما على القصف. اظهرت الاختبارات الجلدية لكل من لقاح tuberculin و لقاحات D.T.P و Tetanus toxoid و الحصبة نتائج سالبة بين المتعرضين بنسبة (62,5٪) مقارنة بغير المتعرضين اذ كانت النتائج سالبة لتلك القاحات (0.00٪). كان التعداد الكلى للبيضاويات طبيعيا للمتعرضين بنسبة (70٪) فى الوقت الذى كانت نسبة الخلايا اللمفاوية دون المستوى الطبيعى عندهم بنسبة (80٪). كل الحالات التى اظهر نتائج سالبة للفحوصات الجلدية كان معدل الخلايا اللمفاوية لديهم دون مستواه الطبيعى مما يدل على ان هنالك خللا المناعة الخلوية لديهم.

لقد اظهرت فحوصات قياس الاضداد بأن IgG و IgA كانا دون المستوى الطبيعى عند المتعرضين بنسب 12.5٪ و 52.5٪ على التعاقب فى حين كانت نسبة IgM اعلى من المستوى الطبيعى بين 22.5٪ من المتعرضين , بينما كل النتائج التى تم الحصول عليها الغير لمتعرضين كانت طبيعية. تظهر هذه النتائج بان المناعة الضدية للمتعرضين تعانى من الخلل ايضا كما كانت عليه المناعة الخلوية.

كانت هنالك فروقا معنوية بين المتعرضين و غير المتعرضين فيما يخص العدد الكى للبيضاويات ($p = 1.1 \times 10^{-4}$) و الخلايا العدلة ($p = 8.8 \times 10^{-2}$) و الخلايا اللمفاوية ($p = 0.0$) و IgG ($p = 7 \times 10^{-10}$) و **IgA** ($p = 1 \times 10^{-10}$). اثبتت النتائج اللتى تم الحصول عليها استخدام غاز الخردل اذ كان تأثيره مزمنا.

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